Appendix 2.2

Task 2.2: Biological Denitrification

Submitted by:
John Copeland
Nitrate Removal

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ORGAINIZATION(S) THAT PREPARED THIS REPORT

EPRI Community Environmental Center

Nitrate Removal Technologies, Inc.

CITATIONS

This report was prepared by

EPRI Community Environmental Center
Washington University
Campus Box 1150
St. Louis, MO 63130

John Murphy
Kim Shilling

This report describes research by EPRI, Southern California Edison, and the California Energy Commission.
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REPORT SUMMARY

This report analyzes the use of a revolutionary, biologically based treatment system for the removal of nitrates from drinking water sources.

Background

The City of Modesto treats water from various groundwater sources. Like much of the Central Valley, some of the City's groundwater sources are contaminated with nitrates as the result of the Valley's historically extensive cultivation. In all likelihood, this problem will continue to worsen. Unfortunately, conventional technologies for nitrate removal are expensive and, often, challenging to operate. Thus, there is a tremendous need for low-cost, easily operated nitrate treatment technologies.

The City of Modesto, Nitrate Removal Technologies, the California Energy Commission (CEC) and EPRI funded and managed a pilot study to evaluate biological denitrification as a viable solution to the nitrate contamination problem now facing many public water supplies throughout the United States. The pilot plant facility will be located in Modesto, California. The demonstration study will include the determination of EPA and California drinking water regulations. NRT has worked with the California Department of Health Services (DHS) and the City of Modesto in the development of a work plan and monitoring schedule for the demonstration system. The specifications for the system and the monitoring schedule for the demonstration test were developed to ensure that the treatment system meets or exceeds current drinking water quality requirements.

Objectives

The project has four objectives:

- Demonstrate the *BioDen*™ biological denitrification system is technically and economically viable at scale-up.
- Obtain California DHS approval for the *BioDen*™ system as a viable treatment system for the removal of nitrate and the production of potable water.
- Demonstrate the effectiveness of ceramic filtration as a viable post treatment filtration technique. .
- Determine operational and maintenance costs for the *BioDen*™ biological denitrification unit using the BASX ceramic microfilter, specifically focusing on power consumption and methods to minimize power requirements during the Phase II demonstration.

Approach

The demonstration project will be separated into three phases: Phase 1 will be a 10-gpm pilot test for two months, Phase II will be a 300 gpm large scale demonstration evaluate for three months, followed by Phase III that will include an additional 300 gpm treatment capacity to complete the installation as a commercial plant. Final design details, operational procedures and overall treatment ability of the system will be determined during Phase I.

Project Status

In 1995, the City of Modesto purchased the Grayson water system from Del Este. The water supply is valuable to the City, but the water is contaminated with excess nitrates. Shortly after the purchase, US Filter, a major vendor of water treatment equipment, recommended the purchase of a reverse osmosis treatment system for water from the Grayson system. However, City staff encountered problems in developing acceptable plans for disposal of the brine, so the idea was scrapped and alternatives were sought. Since that initial assessment, the City has considered drilling new wells, implementing ion exchange, and installing new water lines to facilitate blending of water.

Each conventional alternative has various advantages and disadvantages. Thus, the City is interested in assessing biological denitrification as a low-cost option to the more conventional possibilities. Significant headway was made in implementing the demonstration project described in this report. However, at the end of 2000 the City decided to delay the demonstration project in favor of drilling a new well approximately 1,000 feet deep to obtain water with nitrates below the action level. It is the hope of EPRI and the researchers that the demonstration project is launched in the next 12 to 18 months.

EPRI Perspective

EPRI's Municipal Water and Wastewater Program was created to help member utilities address the energy needs of the more than 60,000 water systems and 15,000 wastewater systems in the United States. Theses facilities are among the country's largest energy consumers, requiring an estimated 75 billion kWh nationally, about 3 to 5 percent of the annual U.S. electricity use.

Interest Categories

E3003 Waste & Water Management
L3004 Municipal Water & Wastewater

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EXECUTIVE SUMMARY

Project Overview

The City of Modesto, Nitrate Removal Technologies, the California Energy Commission (CEC) and EPRI funded and managed a pilot study to evaluate biological denitrification as a viable solution to the nitrate contamination problem now facing many public water supplies throughout the United States. The pilot plant facility will be located in Modesto, California. The demonstration study will include the determination of EPA and California drinking water regulations. NRT has worked with the California Department of Health Services (DHS) and the City of Modesto in the development of a work plan and monitoring schedule for the demonstration system. The specifications for the system and the monitoring schedule for the demonstration test were developed to ensure that the treatment systems will meet or exceed current drinking water quality requirements.

The demonstration project will be separated into three phases: Phase 1 will be a 10-gpm pilot test for two months, Phase II will be a 300 gpm large scale demonstration evaluate for three months, followed by Phase III that will include an additional 300 gpm treatment capacity to complete the installation as a commercial plant. Final design details, operational procedures and overall treatment ability of the system will be determined during Phase I.

Purpose of Project

With the increasingly difficult prospect of supplying high-quality drinking water in areas contaminated with nitrate, such as the current situation in many areas within California, the need for cost-effective nitrate removal solutions is critical. This demand for effective nitrate removal technologies has increased the interest and acceptability of biological denitrification as an attractive treatment solution to municipalities with source waters currently exceeding the nitrate Maximum Contaminant Limit (MCL) of 10 mg/L as nitrogen. This project will utilize the patented *BioDen*™ nitrate removal process. A small municipality in Oklahoma has operated this technology successfully for over two years using slow sand filtration. However, the use of this technology for wells in California requires the evaluation and approval by the California DHS. This evaluation requires modifications to the filtration process within the system, as dictated by DHS, as well as long-term performance monitoring. Therefore, the main purpose of this project is to overcome regulatory and municipality concerns by using membrane filtration technology to provide biologically stable water.

For several years, the City of Modesto has been operating-at a considerable loss- an electrodialysis unit to reduce nitrate level in the water supplied to the residents of the Grayson housing development. The City wishes to find a less expensive, easy-to-operate treatment process with little or no waste discharge.

Project Goals

The project has four, principal goals:

- Demonstrate the *BioDen*™ biological denitrification system is technically and economically viable at scale-up.
- Obtain California DHS approval for the *BioDen*™ system as a viable treatment system for the removal of nitrate and the production of potable water. To do this we will test and monitor the *BioDen*™ system by collecting and analyzing data and operational parameters as it pertains to both chemical and biological standards for finished water quality.
- Demonstrate the effectiveness of ceramic filtration as a viable post treatment filtration technique. This will be done by comparing the BASX ceramic microfiltration technology with a baseline established with a conventional slow sand filter during the Phase I pilot test.
- Determine operational and maintenance costs for the *BioDen*™ biological denitrification unit using the BASX ceramic microfilter specifically focusing on power consumption and methods to minimize power requirements during the Phase II demonstration.

Pilot Test

An on-site pilot test will be conducted to accomplish the following:

- Determine the final design criteria for the 300 gpm Phase II demonstration equipment based upon actual water and operating parameters at the Grayson site.
- Provide test data to enable the Phase II demonstration period to be shortened to 90 days. This will significantly reduce the cost and waste of water during the Phase II demonstration.
- Shorten the startup period of the Phase II demonstration equipment by more closely determining the optimum operating parameters.
- Provide the baseline for comparing the BASX ceramic microfiltration technology with that of a conventional slow sand filter.

During the initial pilot test during Phase I, a ceramic membrane filter preceded by UV disinfection and a slow sand filter will be used for final filtration. The slow sand filter will be used as a baseline filter for final evaluation of the ceramic membrane filter system. After the Phase I pilot test is completed, only the ceramic membrane filter system will be used. Results from the use of UV disinfection prior to the ceramic membrane in Phase I will determine whether it will be used in Phase II. This use of UV is not for a disinfection credit but rather is to determine the effectiveness of UV in enhancing the performance of the ceramic membrane filter.

Demonstration System

The Phase II demonstration system utilizes a series of unit operations that efficiently remove nitrates from the well water while minimizing system complexity and operation. This demonstration system will consist of biological denitrification reactors, a roughing filter (pre-filter), a ceramic microfiltration system, and a chlorine disinfection system. In addition, a PLC will be used to control blending of treated water, the dosing of carbon (external energy source), disinfection, and to perform monitoring and data logging of nitrate, nitrite, total organic carbon, and turbidity throughout the system.

The filtration polishing system is designed as a multiple barrier process that will meet effluent water quality goals while also producing safe potable water. Specifically, the multi-barrier treatment for biological contaminants such as bacteria and viruses includes an "absolute barrier" filtration using ceramic microfilters with a 0.2 um pore size, and further deactivated by chlorine disinfection.

Regulatory Status

NRT and the City of Modesto have reached an agreement with the California DHS regarding the overall workplan, including testing and monitoring protocols. Thus, important background efforts are complete which will speed the regulatory approval process necessary to accelerate acceptance of the technology with drinking water professionals.

Project Status

Extensive microbiological characterizations were made of effluent from a biological denitrification demonstration system in Suffolk County, New York in 2000. A summary of those characterizations is included in this report and conclude that no pathological bacteria are formed in the reactors. Further, the majority of the bacteria in the reactors are common nitrifiers, such as *Psuedomonas*, which are ubiquitous in nature.

Although the City of Modesto and NRT have worked closely together during the past two years, the City is unable to commit to complete this project in a timely manner. The delay in implementation of this project is based on the City's belief that they should investigate drilling an 800 – 1,000 ft. test well near the Grayson well site. A final decision on the project will be made in the next 12 to 18 months.

The problems encountered by the researchers in implementing this particular technology at the City of Modesto are an excellent illustration of a common concern of this treatment process by drinking water professionals. Since widespread water treatment was implemented in the United States at the beginning of the 20^{th} Century, a bedrock maxim has been to employ processes which remove or inactivate any microbial growth in the treated water. Even though the BioDen system employs disinfection after the denitrification reactor, use of microbes to remove specific pollutants will require a shift in thinking by these professionals. This change in thinking will only be possible if this technology is successfully demonstrated in many locations.

ABSTRACT

The California Energy Commission, EPRI and Nitrate Removal Technologies (NRT) sponsored a pilot study to evaluate biological denitrification as a viable solution to the nitrate contamination problem now facing many public water supplies throughout the United States. The pilot plant facility will be located in the Central Valley near Modesto, California, at the Grayson water wellfield. NRT has worked with the California Department of Health Services (DHS) and the City of Modesto in the development of a work plan and monitoring schedule for the demonstration system. The specifications for the system and the monitoring schedule for the demonstration test were developed to ensure that the treatment systems meet or exceed current drinking water quality requirements.

The pilot study has four, principal goals, including demonstrating the *BioDen*™ biological denitrification system is technically and economically viable at scale-up; obtaining regulatory approval for the *BioDen*™ system as a viable treatment system for the removal of nitrate in potable water; demonstrate the effectiveness of ceramic filtration as a viable post treatment filtration technique; and determine operational and maintenance costs for the *BioDen*™ biological denitrification unit.

INTRODUCTION

Background

The effect of nitrate in potable water supplies has been identified since the 1940's. However, with the common use of fertilizers this problem is becoming much more widespread. Current treatment technologies are expensive to install and complex to operate, so there is a great need for simpler, less expensive alternatives.

In 1997, EPRI released a report summarizing a denitrification process for drinking water supplies. The report describes a promising alternative to more conventional denitrification methods for potable water supplies. Since the release of that report, there has been significant interest by the drinking water industry to assess the efficacy and advantages of this treatment process.

EPRI, along with the technology developer, Nitrate Removal Technologies of Littleton, Colorado, have conducted a series of demonstration studies throughout the United States. This report, developed for the California Energy Commission as part of their Public Interest Energy Research, describes the significance of this technology as it pertains to the great state of California. In particular, this report discusses the significance of the demonstration projects completed to date, along with the separate microbiological assessments conducted, to present the current status of the process and its applicability to the water treatment industry.

California's Nitrate Problem

As one of the principal agricultural products producers in the world, the state of California is no stranger to the benefits accrued from fertilizer use on crop yield. Fertilizers of all types have been partially responsible for the unprecedented yields from farms in the state for the last forty years. Unfortunately, widespread use of fertilizers has had a negative side, the impact of which is just now becoming understood.

Nitrogen is a key component necessary for the growth of plants, and thus for much of today's cultivated crops. Excess nitrogen has been added in the past in the form of anhydrous ammonia. The risks associated with too little nitrogen are immediately apparent to most farmers (at least within the course of a single growing cycle). The risks associated with applying too much nitrogen, unfortunately, are less obvious.

Today, fifty-five years after the end of World War II and the beginning of widespread use of fertilizers, nitrate contamination of groundwater has become a nationwide problem. One estimate claims that farmers apply 8 million pounds of fertilizer to their crops than is actually used. In the end, most of this material leaches into groundwater where the nitrogen is ultimately converted to nitrates.

With a significant agricultural industry, the state of California has an extensive nitrate contamination problem. According to the Environmental Working Group of Washington, D.C., as many as 8.9 million California's get their water from supplies contaminated with levels of nitrate in excess of the USEPA limit of 10 mg/L as N.

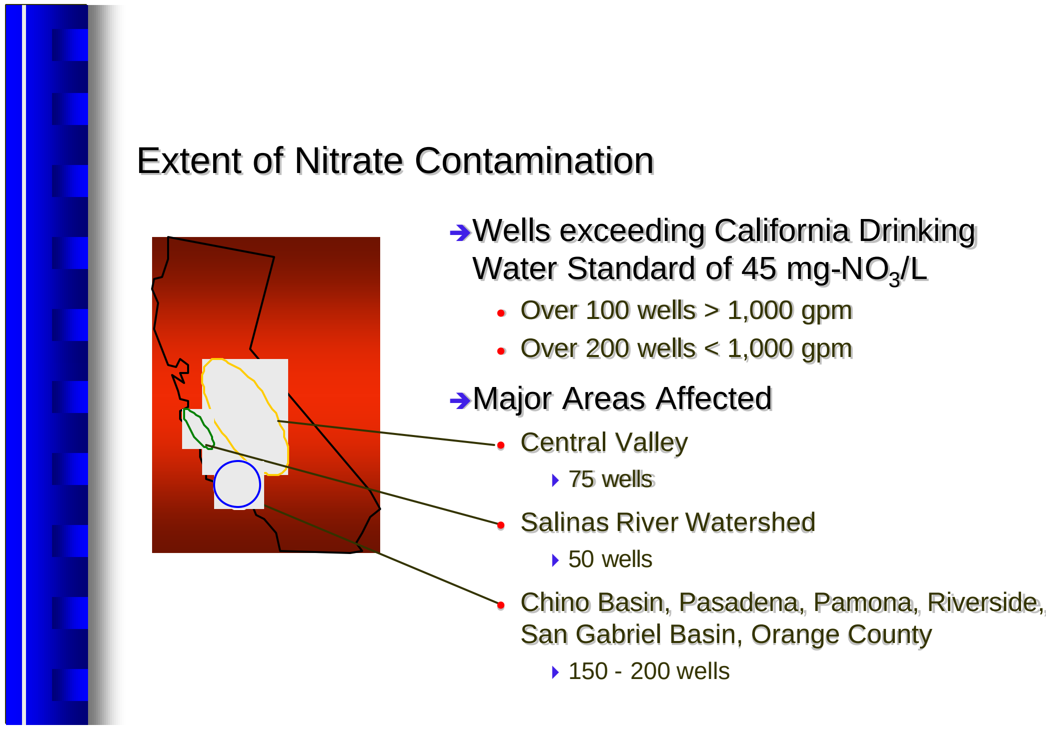

Figure 1. California's Nitrate Problem

Figure 1 delineates those locations within California with the most serious problems with nitrates. There are over 100 large capacity wells (producing more than 1000 gpm) which currently exceed the state's nitrate limit. There are over 200 smaller sized wells with the same problem. The problem is widespread, too, and particularly a problem in the Central Valley, the Chino Basin, and throughout Southern California.

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Conventional Solutions

Traditional Options

In the past, nitrate was removed from drinking water supplies using some form of ion exchange, reverse osmosis, and electrodialysis. Each of these three processes is effective at removing nitrates, but each has significant disadvantages. In ion exchange, the water is pumped through a special medium where a chemical species is exchanged for nitrates absorbed on the media. Under reverse osmosis, the nitrates (and all other ionic species) are essentially strained from the water. Electrodialysis uses an electrical energy to drive the nitrate through special membranes to cathodic plates.

All three processes require significant sophistication to operate. This sophistication is typically much greater than what is currently used by most small system operators in the U.S. Perhaps the most significant problem with these alternatives, however, is the wastestreams generated by each of these processes. In all cases, the wastewater from these processes is a concentrated brine that is very difficult to dispose. Currently, in-land disposal of brine is usually limited to discharge to the local wastewater treatment plant, discharge to an evaporation pond, or deep well injection. All three options are costly in terms of either land cost or operating costs.

The other option most often used by drinking water systems has been to obtain alternative sources. Oftentimes, this entails a significant capital investment in pipelines and new wells or water treatment plant intakes. This option can complicate treatment of a utility's drinking water if the new source has a significantly different chemical make-up than the existing supplies. In addition, the costs associated with new pipelines, easements, permitting and appertunances can be very high.

Given the tremendous difficulties of removing nitrate from potable water supplies using conventional treatment processes, a potentially attractive alternative was investigated by researchers from the University of Colorado. This alternative uses a process common to wastewater treatment to remove nitrates, and is known as biological denitrification.

California's Options

Throughout California, nitrate removal options are similar to those described above for the rest of the U.S. However, many water suppliers prefer blending contaminated supplies with low-nitrate water. A summary of the costs of the various options is included in Table 1.

Thus, the majority of Californian water suppliers try to purchase low-nitrate water, drill new wells, or adopt more traditional treatment technologies. Purchasing low-nitrate water costs from $ 200 to $ 500 per acre-foot, but prices are expected to continue to rise as California's population grows. Drilling new wells is often not a viable option because, given the widespread nature of the problem, new well sites are either unavailable or are located a vast distance from the point of use, making this option uneconomical.

Nevertheless, there continues to be widespread reluctance among drinking water professionals to use microbial techniques in achieving drinking water treatment goals. The speciation described in subsequent chapter was the result of one water utility's reluctance to demonstrate this technology for fear that the microbes could cause problems for both the utility's staff and customers. Interestingly, the speciation work established that the nitrifiers grown in the denitrification reactor are common to the environment and not pathogenic to humans.

Table 1: Summary of Nitrate Treatment Options for California

Per 1,000 gallons	*Capital Costs*	*Operating Costs*	*Brine Disposal*	*Total Cost*
RO	$ .44 - .88	$ 1.10 - 3.00	$ .40 - 2.60	$ 1.54 - 6.48
Ion Exchange	$ .24 - 1.18	$ .46 - .64	$ .04 - .32	$ .70 - 2.14
Water Purchase	Varies	$ .Varies	NA	$ .50 - 1.84
BioDen	$ .40 - .90	$ .50 - .80	$ .01 - .02	$ .91 - 1.72

BIOLOGICAL DENITRIFICATION PROCESS DESCRIPTION

Overview

Biological denitrification is the process of using common nitrifying bacteria under anaerobic (i.e. without oxygen) conditions to remove nitrates from groundwater supplies. The denitrification requires the bacteria grow on a stable medium and are fed a carbon source. The bacteria then use the nitrogen in the nitrate for respiration, converting it from nitrate to gaseous nitrogen.

In wastewater treatment, other associated organic compounds in the wastewater are used by the nitrifiers as a carbon source. Groundwater, on the other hand, is largely devoid of any carbon source, so this process requires adding a carbon source of some type. Food-grade acetic acid (i.e. vinegar) is an excellent material for purpose in potable water treatment. Unfortunately, it is very expensive, so University of Colorado researchers settled on very low concentrations of corn syrup.

Thus, the biological denitrification treatment process is simply a matter of promoting the proper conditions and letting nature takes it course. Corn syrup is added to the raw water, which is pumped through a very large tank containing plastic media. Over time, the nitrifiers proliferate and consume the levels of nitrates in the groundwater.

The treated water is then pumped through a filter to remove any bacterial growth that sloughs off from the reactor and adding a disinfectant.

EPRI has partnered with Nitrate Removal Technologies to further develop and promote biological denitrification of potable water supplies. To better test the concept on various water supplies, NRT has built a portable pilot plant. This system has been tested in a number of locations. The process is shown in Figure 2.

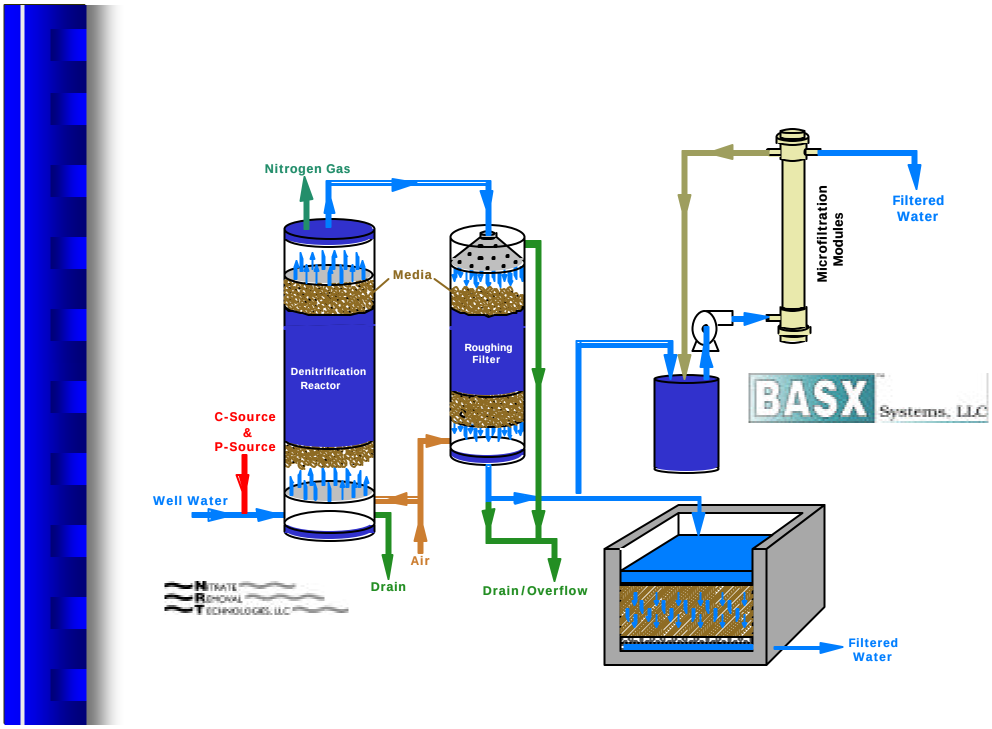

Figure 2. Nitrate removal process schematic.

Filtration Alternatives

A recently completed study in New York State considered the comparison of four filtration technologies for the biological denitrification process. The four filters considered include slow sand filtration, which is most commonly recommended for the biological denitrification process, a bag-and-cartridge filter, and two types of filter modules.

The four systems offer different advantages and disadvantages. Slow sand filtration, where the water passes slowly through a bed of sand at a very low loading rate, is an excellent alternative for small treatment systems. Slow sand filters remove particles and achieve a modest degree of biological treatment. However, these are inappropriate for large systems due to the large amount of surface area needed and the labor-intensive nature of maintaining the filters.

The filter modules and bag-and-cartridge filters are more suitable for larger systems. These filters will not provide any biological treatment, but can remove particles down to a specified size. This operating characteristic, coupled with their availability in a range of sizes, makes them quite suitable for treating large flows. The New York pilot study was conducted on Long Island, so there was a need to evaluate filtration systems capable of treating large flows.

The researchers in the New York study determined that while three of the four filtration options are viable, the two filter modules, including a hollow fiber configuration and a ceramic microfilter, were somewhat susceptible to biofouling. Without a small chlorine residual, both systems tended to biofoul very quickly. On the other hand, the bag-and-cartridge filter was clogged very quickly. Pressure across the cartridge, known as transmembrane pressure or TMP, increased to unacceptable levels after only three to four hours of operation. At these unacceptable rates, the bag-and-cartridge filter was deleted from testing protocol and no longer considered.

Process Summary

The detailed filtration alternatives assessed in the study in New York State focused selection of the filters for the demonstration plant in Modesto. Clearly, both the filter modules and the slow sand filters are acceptable for use at Modesto. However, given the very high flow rates anticipated in the full-scale demonstration system during the second phase of testing, it was concluded that during the Modesto tests only the two filter modules would be used.

The demonstration tests at Modesto will include a roughing filter, the denitrification reactor module, and two different filter modules. The choice of filter modules has not been determined, but it is anticipated that at least one of them will be identical to one of the two tested in New York State. A second module will be chosen to expand the treatment database.

As the pilot plant design was nearing completion, concerns were voiced by the Modesto staff over the use of microbes in the potable water treatment process. These concerns, which were also voiced during the study in New York State, are quite valid given the drinking water industry's historical concern with pathogenic organisms present in water supplies. The results of the characterization studies completed in New York State are included in this status report in order to ease some of these fears. That discussion is summarized in the following chapter.

MICROBES IN POTABLE WATER TREATMENT

This use of microbes in potable water applications is a new concept, so there is understandable concern among drinking water professionals charged with protecting the public health from dangerous pathogens. Thus, it is worthwhile to summarize the microbiological quality in conventional water treatment and the results developed from a characterization of the microbes in the BioDen system that was completed during the pilot assessment in New York State.

CONVENTIONAL WATER TREATMENT PLANT MICROBIOLOGICAL QUALITY

SOURCE WATERS

The evaluation of microbial quality of drinking water sources is limited. There is no specific occurrence data for individual microorganism species except for *Giardia, Cryptosporidium,* and a handful of viruses and bacteria. Due to the increased concern of pathogenic microorganisms within finished drinking water, the EPA has developed a Drinking Water Contaminant Candidate List that includes 10 microbiological contaminants. In an effort to better understand the occurrence of bacteria in the drinking water sources the EPA has initiated the Information Collection Rule (ICR) to collect information to support development of national drinking water standards. The ICR data collected and posted by the EPA to date (July-97 through December-97) is summarized in Figure 3 for total coliform, fecal coliform, and *E. coli* bacteria. The data collected is for water sources including both surface water and groundwater.

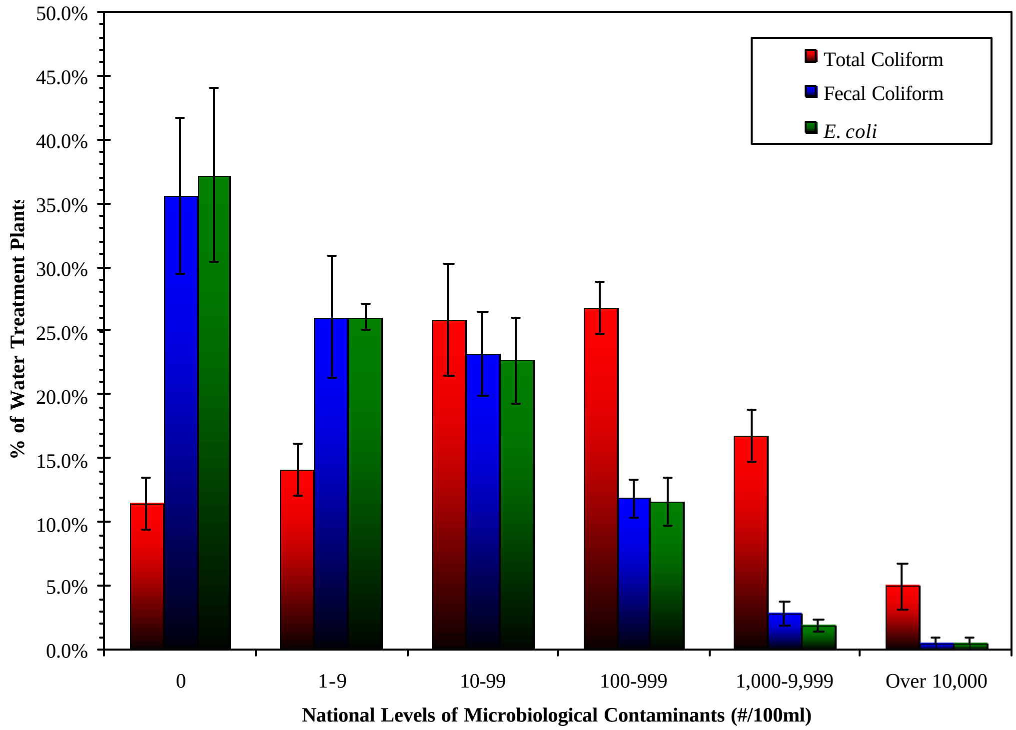

Figure 3. National Levels of Microbiological Contaminants

The data summarized in Figure 3 indicates that over 88% of all source waters for the sampled utilities had coliform bacteria present with 48% of the systems having coliform levels greater than 100 cfu/100 ml. Over 65% of the systems sampled were positive for fecal coliform or E. coli bacteria with 14% of the systems having fecal coliform and *E. coli* levels greater than 100 cfu/100 ml. This data suggests that coliform bacteria are present in the majority of source waters used for drinking water treatment with an alarming number having fecal coliform as well.

FILTER BEDS/WATER PLANT EFFLUENT

Typically filtration is the last physical process before disinfection. There is little information on the number and types of bacteria that inhabit filters in conventional treatment plants and in plants utilizing biological active filtration (BAF). However, there is limited data on the microbiological characteristics in the effluent of a handful of water treatment facilities. Table 2 summarizes organisms that were found in three water plant effluents after disinfection (adapted from Geldreich et al., 1977).

Table 2: Microorganisms Identified in Water Plant Effluents

Significant Categories	Isolated Organisms
Total Coliform	*Klebsiella Pneumoniae* *Enterobacter cloacae* *Erwinia herbicola*
Coliform Antagonists	*Pseudomonas fluorescens* *Pseudomonas maltophila* *Flavobacterium* sp.
Opportunistic Pathogens	*Moraxella* sp. *(sA)* *Staphylococcus*
Other	*Acinetobacter calcoaceticus* *Neisseria flavescens*

The bacteria listed in Table 1 are all chemoorganotrophs with the majority being facultative anaerobes. The coliform antagonists *Pseudomonas flourescens* and *Pseudomonas maltophila* are animal pathogens (see Table 4). Other bacteria within the table are pathogenic with others being normal flora in the human mouth (Neisseria), skin (Acinetobacter, Staphylococcus), the respiratory tract (Neisseria, Staphylococcus) and the urogenital tract (Klebsiella, Neisseria). The results in Table 1 must be used with caution due to the fact that data is not current (1977). However, the data illustrate that various bacteria including pathogens may be present in water treatment plants even before adequate disinfection.

GRANULAR ACTIVATED CARBON (GAC)

It has long been considered that biological activity on GAC is beneficial in that specific compounds can be removed by biological oxidation rather than by adsorption. However, GAC generally causes the concentration of microorganisms in the GAC column effluent to increase compared to the influent water. This relationship holds for both total coliform analysis as well as HPC analysis. The increase in bacterial number can be quite significant. At a plant in Beaver Falls, PA, coliform bacteria including *Citrobacter freundii*, *Enterobacter cloacae*, and *Klebsiella pneumonia* were isolated in the effluent (Water Quality and Treatment, 1990). Even with the presence of these coliform bacteria, the system met all EPA regulations after post-disinfection.

DISTRIBUTION SYSTEMS

Historically, most, if not all, waterborne disease outbreaks have been linked to pathogenic organisms with origins traced to fecal matter from warm-blooded animals and humans. Recently, however, evidence has indicated that another source of pathogens is the water distribution system and associated storage facilities. Breaks in the system can allow the introduction of pathogens into the system as well as many bacteria can grow and persist within distribution systems depending on disinfection practices. Table 2 summarizes the types of coliform bacteria that were identified in 111 public water supply distribution systems.

Table 3: Microorganisms Identified in Distribution Systems*

Significant Categories	Isolated Organisms
Total Coliforms	*Klebsiella Pneumoniae* *Enterobacter cloacae* *Eneterobacter herbicola* *Enterobacter aerogenes* *Escherichia coli* *aeromonas hydrophila* *Cirobacter freundii*
Coliform Antagonists	*Pseudomonas fluorescens* *Pseudomonas maltophila* *Flavobacterium* sp. *Pseudomonas cepacia* *Pseudomonas putida* *Pseudomonas aeruginosa* *Bacillus* sp. *Actinomycetes* sp.
Opportunistic Pathogens	*Moraxella* sp. *Staphylococcus* *Klebsiella rhinoscheromatis* *Serratia liquefaciens* *Serratia marcescens*
Other	*Acinetobacter calcoaceticus* *Streptococcus* sp. *Corynebacterium* sp. *Micrococcus* sp. *Nitrococcus* sp.

* adapted from Water Quality and Treatment, 1990

Among the coliform bacteria, encapsulating bacteria such as *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Citrobacter freundii* are the most successful colonizers. In addition to coliform bacteria in distribution systems, many other bacteria, such as *Legionella*, have also been isolated from various systems (Lin et al., 1998).

In addition to the bacteria listed in Table 2. *Mycobacterium avium* complex (MAC) has been observed in numerous distribution systems throughout the US (AWWA Microbiological Contaminants Research Committee, 1999).

This new evidence of microbiological quality in distribution systems along with increased analytical methods, have increased the awareness of potential new pathogens along with common pathogens, such as coliform and *E. coli*, in water systems. This is evidenced by the fact that in many recent reports reviewed and commented upon by water treatment providers, consultants, academicians, and regulatory agencies, the number one concern was microbial quality and emerging pathogens in drinking water (Opflow, 1999).

The presence of bacteria in distribution systems, such as that presented in Table 2, appears to be very common with potentially significant numbers of both coliform and pathogenic bacteria.

MICROBIOLOGICAL ASSESSMENT OF THE *BIODEN*™ SYSTEM

Biological Denitrification – Overview

Denitrification is the biological (bacterial) conversion of nitrate to harmless nitrogen gas. Biological denitrification is an aerobic respiration process where nitrate acts as the terminal electron acceptor while an external carbon source is the electron donor.

Bacteria

Several genera of bacteria can denitrify including *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Brevibacterium, Falvobacterium, Lactobacillus, Micrococcus, Proteus, Psuedomonas*, and *Spirillum.* This large diversity and number of bacteria that can denitrify is due to the fact that the enzymatic pathway for nitrate reduction can be achieved by modifications within the bacterial cell. The large number of bacteria that can denitrify also means that denitrification is a relatively stable process and is possible in a wide range of environmental conditions.

As stated previously, biological denitrification by bacteria requires an external carbon source for energy. As stated in previously, by definition biological denitrification is carried out by heterotrophic (chemoorganotrophic) bacteria. In denitrification, dissimilatory nitrate reduction occurs in the following steps:

$$NO_3 \Rightarrow NO_2 \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2$$

This multi-step reduction is a direct result of the cytochromes within the electron transport chains (ETC) inside the bacterial cell, although the biochemistry of these reactions is not well understood.

Stoichiometry

The objective in denitrification is the removal of nitrate (electron acceptor) by adding a sufficient amount of carbon substrate (electron donor). This requires knowledge of both the kinetic and stoichiometric requirements of the system. The stoichiometry of biological denitrification is dependent on the type of external carbon (supplemental) source used. Various reduced-carbon sources have been used including high fructose corn syrup (HFCS), ethanol, methanol, acetic acid, and denatured alcohol. Stoichiometric equations can be developed by both theoretical and laboratory investigations. Once the stoichiometric equation is known, then the amount of carbon required to destroy the nitrate ion can be determined.

For example, if acetic acid is used as the carbon source and nitrate is the nitrogen source for cell growth, then the overall stoichiometric equation is (assuming 65% is used for energy production and 35% is converted to cell mass):

$$1.0\ NO_3^- + 0.88\ CH_3COO^- + H^+ \Rightarrow$$
$$0.088\ C_5H_7NO_2 + 0.46\ N_2 + 0.42\ CO_2 + 0.88\ HCO_3^- + 1.07\ H_2O$$

This stoichiometric equation indicates that for every mg of nitrate (NO_3^-) destroyed, approximately 1.5 to 1.6 mg of carbon is required (ratio 1.5:1).

Operational and Environmental Variables

Operational and environmental variables that affect denitrification include:

- Nitrate concentration
- Nitrite concentration
- Dissolved oxygen
- Temperature
- pH
- Ionic strength

In addition to those variable listed above that can affect denitrification performance, two micronutrients in particular are very important to denitrification performance. Those micronutrients are Vanadium and Molybdenum. These micronutrients are used in the production of cytochromes and enzymes specific for denitrification within the cell.

BioDen™ Process

The *BioDen*™ process uses bacteria in conjunction with acetic acid (vinegar) to remove nitrates from water. This is an anaerobic biological process in which nitrates (NO_3^-) are converted by bacteria into harmless nitrogen gas (N_2) and carbon dioxide (CO_2). The bacteria that are used in the *BioDen*™ process are naturally occurring, non-pathogenic bacteria. They work within reactors, growing on plastic media in the form of a biofilm. This conversion of nitrate to nitrogen gas within a biofilm is shown in Figure 4.

The *BioDen*™ process uses a mixed population of facultative heterotrophic bacteria to destroy the nitrate molecules. Typically, the bacteria used to inoculate the *BioDen*™ reactor system are naturally occurring bacteria that are cultured and enriched from non-contaminated (fecal) soils. NRT cultures bacteria from the water system's local area in an effort to increase denitrification predictability and reliability within the system.

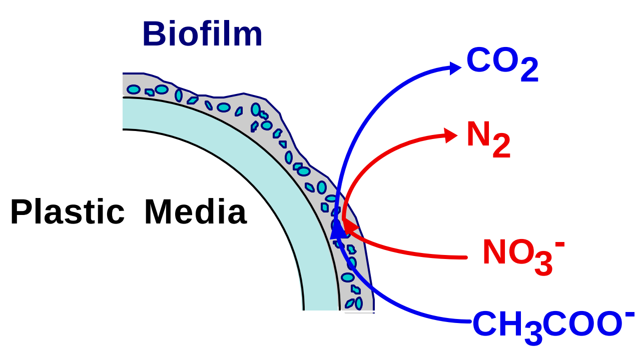

Figure 4. Denitrification Within a Bacterial Biofilm

BioDen™ Denitrification Reactor

The destruction of nitrates is carried out within biological denitrification reactors that incorporate a fixed-film process (Figure 5).

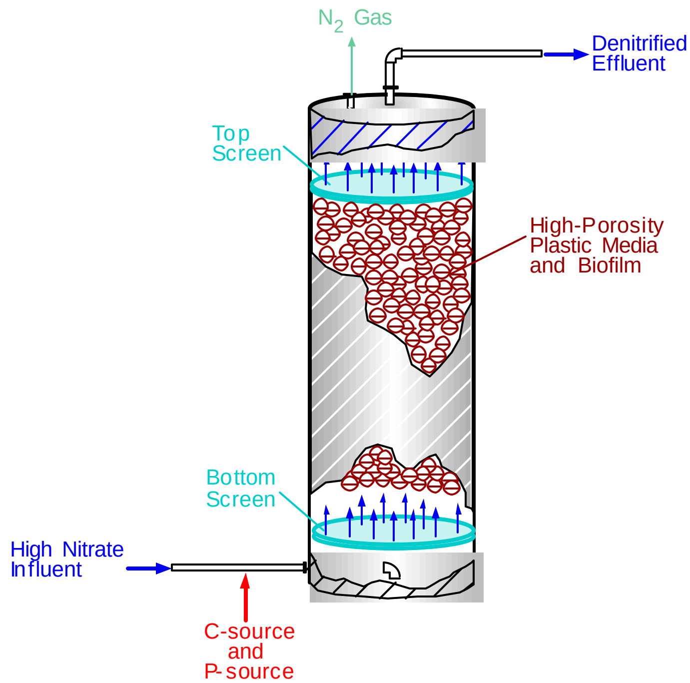

Figure 5. *BioDen*™ biological denitrification reactor

After the addition of an external carbon and phosphorous source, the raw water flows through reactors filled with a high-porosity, low-density packing material. This packing material provides a large surface area for bacterial attachment while minimizing potential channeling and clogging problems. Additionally, flow through the reactor is upward through the reactor to maximize nitrogen gas (N_2) removal. As the water passes through the column, oxygen is first consumed by facultative bacteria followed by the removal of nitrates. Although dissolved oxygen can interfere with denitrification performance, the presence of the proper amount of external carbon allows for the rapid consumption of oxygen and the efficient removal of nitrate. Nitrates diffuse into the attached biofilm and are subsequently converted to nitrogen gas, which is then vented harmlessly out the top of the reactor.

Reactor Growth Environment: Impact on Bacterial Speciation

The growth environment in the *BioDen*™ biological denitrification reactor can be formulated by understanding the biochemical reactions (and chemical reactions) occurring within the reactor. It is assumed that the water entering the reactor has high nitrate, dissolved oxygen (DO), adequate supplemental carbon, and all essential micronutrients necessary for optimal denitrification. It is important to understand the bacterial growth environment within the reactor system because the growth conditions will affect not only the type, i.e. chemoorganitrophic for example, but also the species of bacteria, i.e. pathogens versus non-pathogens.

The growth environment in the biological denitrification reactor used within the *BioDen*^{TM} process is illustrated in Figure 6.

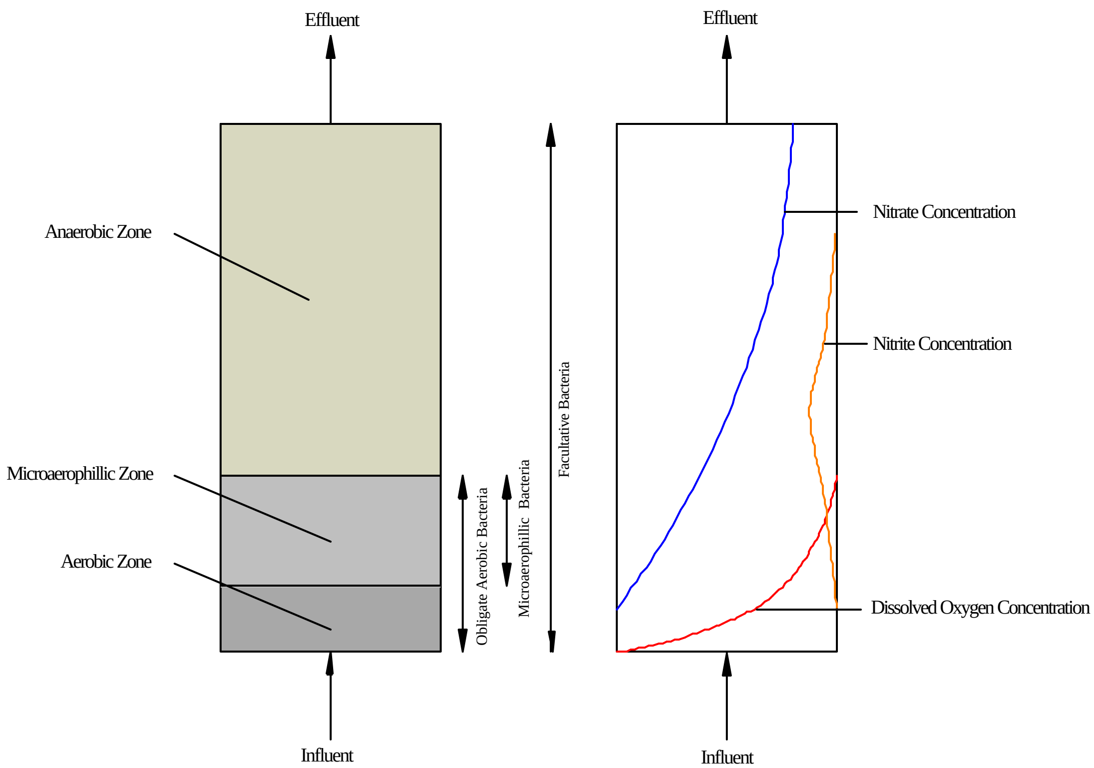

Figure 6. Estimated reaction zones within a *BioDen*^{TM} biological denitrification reactor.

The information represented in Figure 6 is estimated based upon indirect and direct results from various systems and pilot units. The environmental conditions within the reactor are approximate. It is assumed that oxygen will be used most rapidly in the lower zones of the reactor due to theoretical thermodynamic relationships in that bacteria will preferentially use oxygen as a terminal electron acceptor over nitrate. Denitrification can occur within the system even though oxygen is present. This is due to the fact that in fixed film processes incorporating “deep” biofilms, nitrate can diffuse into the lower layers of the biofilm and be reduced even with oxygen present since oxygen cannot diffuse into the deeper parts of the biofilm. The production and subsequent destruction of nitrite is highly dependent on the amount of carbon added, i.e. carbon to nitrogen ratio, and on pH.

The reactor can be conceptually separated into three zones: an anaerobic zone, a microaerophilic zone, and an aerobic zone. These environmental “zones” directly impact the type of bacteria that can survive within each zone and within the entire reactor. As illustrated, only obligate aerobic bacteria can survive in the aerobic zone while microaerophilic bacteria can only survive in a narrow band where the dissolved oxygen concentration is acceptable for growth. In contrast, facultative bacteria, which can use either oxygen or nitrate as an electron acceptor, can survive throughout the entire length of the reactor.

The impact of the environmental growth conditions within the reactor on the potential for pathogen proliferation is summarized in Table 3.

Estimation of pathogen survivability within the denitrification reactor was based upon:

1) Growth conditions for the pathogens of interest.
   The ability to reduce nitrate, food requirements, oxygen requirements, and temperature constraints were used to determine the possibility of growth of the microorganism within the reactor.

2) Probability of pathogen in seed culture.
   This estimation is based upon the fact that the seed culture is enriched from a non-fecal contaminated soil. In addition, the enrichment procedure, which very high nitrate concentrations are used, increases antagonistic interaction where fast-growing denitrifiers will out-compete slower growing denitrifying bacteria and possible pathogens introduced within the soil sample. Also, the probability of having pathogens within the soil sample is analyzed with regards to environmental occurrence of each pathogenic microorganism.

3) Survivability compared to other organisms within the reactor.
   Recent evidence has suggested that the survivability of pathogens (in this study, *Pseudomonas aeruginosa*) is significantly decreased when large numbers of heterotrophic bacteria are present (Denn, 1999). Observations by University and government researchers who have tried to contaminate carbon filters with pathogenic bacteria have shown that pathogen numbers actually decrease (2-log) through the system and eventually die-off entirely. It is theorized that the non-pathogenic heterotrophic bacteria are so well acclimated to the environment that they out-compete the enteric bacteria. This antagonistic interaction may be so significant, that the proliferation of pathogenic bacteria is inhibited, and in fact, are eliminated from the system even if they are introduced into the system.

Using this survivability analysis, Table 3 indicates that there are only 2 bacteria that have an estimated greater than 1 in 5 probability of occurring within the denitrification reactor. Even though coliform bacteria have a high probability of being present within the reactor, the estimated survivability of *Shigella* sp. and *Yersinia enterocolitica* is low because these bacteria are not usually found in soils. The protozoa, viruses, and cyanobacteria listed have an extremely low probability of being present in the reactor.

The bacteria that have the highest probability of being present in the reactor are:

- Total coliforms (non-pathogenic)
- *Pseudomonas aeruginosa* (opportunistic pathogen)

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Table 4: Microorganisms – Presence Probability Within the *BioDen*[TM] Reactor System

Name of Organism or Group	Growth Conditions	Presence Probability[1]
Bacteria		
Campylobacter jejuni	Microaerophilic (3-5% O_2), no nitrate reduction	Very Low
Clostridium perfringens	Strictly anaerobic, some strains can reduce nitrate	Low
Coliform bacteria	Facultative, most can reduce nitrates, 4° to 45°C	High
Enteropathogenic *E. coli*[1]	Facultative, can reduce nitrates, 4° to 45°C	Low
Legionella sp. and related bacteria	Optimal growth 25° to 42°C	Very Low
Mycobacterium avuim[1] Mycobacterium intracellulare[1]	Aerobic, no nitrate reduction, limited growth below 25°C, slow grower	Very Low
Opportunistic bacteria		
Pseudomonas aeruginosa	Facultative, can reduce nitrates, 4° to 43°C	Medium
Aeromonas hydrophila[1]	Facultative, can reduce nitrates, 5° to 41°C	Low
Helicobacter pylori[1]	Microaerophilic, no nitrate reduction	Very Low
Other (atypical) *Mycobacteria*	Aerobic, most are very slow growers, virtually no growth below 25°C	Low
Other *Salmonella*	Facultative, can reduce nitrates, optimal temp. for growth is 37°C.	Low
Salmonella typhimurium	Facultative, can reduce nitrates, optimal temp. for growth is 37°C.	Low
Shigella sp.	Facultative, can reduce nitrates, 4° to 45°C	Low
Yersinia enterocolitica	Facultative, can reduce nitrates, -2° to 45°C	Low
Enteric Viruses		
Adenoviruses[1]	Animal host, can survive in extracellular state	Low
Enteroviruses		
Poliviruses		Very Low
Coxsackie viruses A[1]		Low
Coxsackie viruses B[1]		Low
Echoviruses[1]		Low
Other enteroviruses		Low
Hepatitis A[2]		Very Low
Norwalk and related GI viruses (Caliciviruses)[1]		Low
Reoviruses		Low
Rotaviruses		Low
Protozoans		
Acanthamoeba castellani[1]		Very Low
Balantidium coli	Freshwater and marine, animal parasites, rumen	Very Low
Cryptosporidium[1]	Freshwater, animal parasites	Very Low
Cyclospora cayetanesis[2]	Animal parasite	Very Low
Entamoeba histolytica	Freshwater and marine, animal parasites	Very Low
Giardia lamblia[1]	Fresh water, animal parasites	Very low
Microsporidia[1]	Primarily human parasites	Very Low
Naegleria fowleri		Very Low
Toxoplasma gondii[2]	Primarily animal parasites (insects carry disease)	Very Low
Cyanobacteria (blue-green algae)		
Alphanizomenon flos-aquae[1]	Phototrophic	None
Anabaena flos-aquae[1]		None
Microcytis aeruginosa[1]		None
Schizothrix calciola		None

1- Before filtration and disinfection. Based upon growth conditions within the reactor, probability of organism in seed culture, survivability compared to other probable organisms within the reactor. (very low <1%); (low 1-5%); medium (5% to 20%), high (>20%).

The most important fact from Table 3 is that of the bacteria, in this case pathogenic bacteria, only one opportunistic pathogen and total coliforms are likely to be present. An opportunistic pathogen is a pathogen that rarely infects humans with normal immune responses. Arguably, *Pseudomonas aeruginosa* is the most prolific opportunistic pathogen in the world. It has been identified in approximately 30% of private groundwater wells in one study. The probability of *Pseudomonas aeruginosa* proliferating within the reactor system is less than that for coliform bacteria, approximately 5% to 20%. This lower probability is due to the fact that it has been shown that *Pseudomonas aerugenosa* does not survive in systems were large numbers of non-pathogenic heterotrophic bacteria, such as the *BioDen*^{TM} reactor, are present (Denn, 1999). The probability of coliform bacteria within the reactor system is highest out of all bacteria with a 50% probability because the fact that over 85% of the 250 large water systems monitoring coliform bacteria for the ICR were coliform positive prior to treatment.

Comparing these results to the occurrence of these bacteria in natural source waters and within treatment plants/distribution systems (Section 4 and Section 5), the probability of appearance of these bacteria in the *BioDen*^{TM} reactor effluent is not a large concern. The effect of chlorine on coliform type bacteria and *Pseudomonas aerugenosa* is well documented with very high susceptibilities to chlorine. Therefore, after filtration and chlorination, the system will meet all Federal Drinking water Standards for microbiological quality.

BACTERIA EVALUATION TEST RESULTS

BACTERIA SPECIES IDENTIFICATION

Denaturing gradient gel electrophoresis (DGGE) analysis was used to determine the species of bacteria in the effluent of the *BioDen*^{TM} pilot system located at Suffolk County, New York. The speciation tests identified five dominant species, defined as those bacteria species that compose of at least 1% of the total community of bacteria, within the effluent stream from the *BioDen*^{TM} system. The species include (no specific order):

- *Pseudomonas coronafaciens*
- *Pseudomonas chlorophis*
- *Azospirillum sp.*
- *Zoogloea ramigera*
- *Janthinobacterium lividum*

Bacterial Information

Analysis of the five dominant species within the system shows that none of the identified species are known human pathogens. In fact none are known to be opportunistic pathogens. Phylogenetic and physiological information on the individual species listed above is summarized as:

Pseudomonas coronafaciens

- Cells: straight or curved rods, range 0.5 -1 by 1.5 – 4 μm, gram-negative, strictly aerobic, nitrates not reduced to nitrites or to gaseous nitrogen

This bacterial species is common plant pathogen (phytopathogen) that attacks foliage-causing chlorosis on leaves (Madigan et al., 1997). This bacterium is rarely found free in soil (Madigan et al., 1997). This bacteria has been reclassified as *Pseudomonas syringae.* This bacteria has not been shown to denitrify as defined as no observable denitrification in greater than 90% of all known strains (Bergey's Manual of Determinative Bacteriology, 1994). The probability of this bacteria being present is low although it could be present at very low concentrations (i.e. very low percentage of total bacteria present). This bacteria is not considered a human pathogen.

Pseudomonas chlorophis

- Cells: straight or curved rods, range 0.5 -1 by 1.5 – 4 µm, gram-negative, can denitrify by aerobic respiration, nitrates reduced to nitrites, nitrous oxide, or to gaseous nitrogen

This bacterial species is relatively common soil microbe that is heterotrophic and capable of denitrification (Bodelier et al., 1997). The likeliness of this bacteria being present is high due to the fact that it can denitrify, it is heterotrophic, and was probably present in the soil seed from which the denitrifying bacteria were enriched. This bacteria are not considered human pathogen.

Azospirillum sp.

- Cells: spiral-shaped, range 1 by 2.5 – 3 µm, gram-negative, microaerophilic, nitrogen gas reduced to ammonia with subsequent conversion to organic nitrogen

This bacteria is a common nitrogen-fixing bacterium found in natural soils (Han and New, 1998). These bacteria are typically associated with grasses and legumes. The percentage of these bacteria within the system is probably very low due to the fact that they require oxygen. It is possible for them to be present in the system due to the production of nitrogen gas by the denitrifying bacteria present. They are most likely growing at the bottom of the reactors where the oxygen concentration is highest. This bacteria is not a human pathogen.

Zoogloea ramigera

- Cells: rod shaped, range 0.5 – 1 by 1 – 3 µm, gram-negative, microaerophilic, nitrates can be reduced to nitrites or to gaseous nitrogen

Zoogloea ramigera is considered a slime forming bacteria. It is found in soils and many biological systems (Madigan et al., 1997; Rosselló-Mora et al., 1995). This bacteria is considered to be a "slime" producer even though every bacteria produces EPS (extracellular polymeric substances). Generally, *Z ramigera* is microaerophilic. However, depending on the strain of bacteria, varying environmental conditions can be colonized by Z. ramigera. For example, a strain of Z. ramigera was found to perform axenic nitrate reduction with oxygen concentrations as high as 8 mg/L (Strand et al. 1988). Therefore it is possible that this bacteria may be denitrifying within the reactors. The concentration of this bacteria, however, is probably low due to the fact that it cannot compete well with the Pseudomonad denitrifying species due to growth conditions within the reactor system. This bacteria is not considered pathogenic to humans.

Janthinobacterium lividum

This bacteria is also commonly found in soils. It has been used for the degradation of hazardous materials in 2,4-dinitrophenol (Silverstein, personal correspondence). This bacteria is not considered harmful to humans.

DISCUSSION

Using the information presented above, it can be formulated that the likelihood of the Pseudomonas species identified of being pathogenic is low due to the fact that:

i. *P. coronafaciens* do not denitrify. Due to the environmental conditions within the reactor, i.e. reducing environment, low oxygen, the probability of this bacteria growing is very low.
ii. *P. coronafaciens* is rarely found free in soil. Since the bacterial seed was a soil sediment collected on-site, the probability of being enriched during start-up is very low.

Therefore, it is probable that the predominant denitrifying bacteria within the reactor system is *Pseudomonas chlorophis.* This is consistent with available data showing this bacteria to be very common in soils and its ability to denitrify.

The apparent occurrence of *Azospirillum sp.* is somewhat surprising at first glance due to the fact that this bacteria is microaerophilic and fixes gaseous nitrogen gas (N_2) to ammonia and subsequently to organic nitrogen. Its occurrence can be explained by the fact that the bacteria were probably present in the soil sample used to grow the culture and that nitrogen gas is produced during denitrification. It is likely that their numbers are very low within the reactors. The observable presence of *Zoogloea ramigera* is not surprising since many species are found in the natural soil environment and that bacterial-strains can occupy many different environmental niches. Environmental conditions within the reactor would suggest that *Z. ramigera* present in this system is a strain that can denitrify.

In addition, the results indicate that the bacteria within the reactor are predominantly gram-negative rod or spiral shaped bacteria. The bacteria isolated can occupy various environmental conditions including strictly aerobic, microaerophilic, and facultative conditions.

CONCLUSIONS

The bacterial species identified are not known to be human pathogens. Comparison of the *Pseudomonas* sp. isolated from the system with the known *Pseudomonas* sp. that are pathogenic listed in Table 4, indicates that the *Pseudomonas* sp. bacteria isolated are not pathogenic to humans. Also, the remaining bacteria isolated including *Azospirillum* sp., *Zoogloea ramigera*, and *Janthinobacterium lividum* are not known to be human pathogens.

COLIFORM AND FECAL COLIFORM RESULTS

The most dominant (greater than 1% of total bacterial population) individual species within the *BioDen*^{TM} system are summarized below. In addition to these identifications, bacterial analyses from bench-scale, pilot-scale and commercial installations have indicated that coliform bacteria can vary depending on the carbon source used and the type of filtration media. Coliform and fecal coliform bacteria were analyzed in all installations. Fecal coliform has never been detected. Coliform results from these installations are summarized in Table 5. The combined average of over 25 sample measurements from these studies is 6.0×10^2 ($\pm 3.6 \times 10^2$) coliform bacteria per 100 ml. When comparing this number to the data presented in a previous section where 48%

Table 5: Pathogenic Pseudomonas Bacteria

PSEUDOMONAD SPECIES	RELATIONSHIP TO DISEASE
Animal Pathogens	
P. aeruginosa	Opportunistic pathogen, especially in hospitals; in patients with metabolic, hematologic, and malignant diseases; hospital-acquired infections from catheterizations, tracheotomies, lumbar punctures, and intravenous infusions; in patients given prolonged treatment with immunosuppressive agents, corticosteroids, antibiotics and radiation; may contaminate surgical wounds, abscesses, burns, ear infections, lungs of patients treated with antibiotics; primarily a soil organism.
P. fluorescens	Rarely pathogenic, as does not grow well at 37^{o}C; may grow in and contaminate blood and blood products under refrigeration.
P. maltophilia	A ubiquitous, free-living organism that is a common nosocomial pathogen.
B. cepacia	Causes onion bulb rot; has also been isolated from humans and from environmental sources of medical importance.
P. pseudomallei	Causes melioidosis, a disease endemic in animals and humans in Southeast Asia
P. mallei	Causes glanders, a disease of horses that is occasionally transmitted to humans.
P. stutzeri	Often isolated from humans and environmental sources, may live saprophytically in the body
Plant Pathogens	
P. solanacearum	Causes wilts of many cultivated plants.
P. syringae	Attacks foliage, causing chlorosis and necrotic lesions on leaves; rarely found free in soil.
P. marginalis	Causes soft rot of various plants.
Xanthomonas	Causes necrotic lesions on foliage, stems, fruits; rarely found free in soil.

Adapted from Madigan et. al. (1997).

Table 6: Coliform Levels in *BioDen*^{TM} Reactor Effluent

Installation	Coliform Level	Reference
UC laboratory work	5.0×10^{2} ($\pm 3.7 \times 10^{2}$)*	Cook et. al., 1991
UC pilot demonstration	6.9×10^{2} ($\pm 4.8 \times 10^{2}$)	Cook and Silverstein, 1989
Coyle, OK commercial installation	5.8×10^{2} ($\pm 2.0 \times 10^{2}$)	NRT internal document
Combined Average	6.0×10^{2} ($\pm 3.6 \times 10^{2}$)	

* One standard deviation from average value.

of the sampled water systems for the ICR had bacterial levels greater than 1×10^{2} coliform bacteria per 100 ml, the effluent from the *BioDen*^{TM} system does not have overly excessive coliform bacteria levels. In addition, with pre-filtration and final filtration polishing using microfiltration, the probability of any coliform bacteria present in the final chlorinated water is very low and will meet all Federal Drinking Water Standards for microbiological contaminants during normal disinfection operations. With regards to fecal coliform and *E. coli*, the *BioDen*^{TM} system compares very favorably when considering that over 65% of the sampled water systems for the ICR had fecal coliform and E. coli bacteria present. The effluent from the *BioDen*^{TM} reactors has never had fecal coliform or *E. coli* present.

PROJECT STATUS SUMMARY

Technology Development

As of early 2001, the EPRI Municipal Water and Wastewater Program has sponsored or managed three different assessments of this technology that have proven its effectiveness. This section summarizes that developmental work

The initial development of this technology centered around a study by researchers from the University of Colorado in Wiggins, Colorado. That pilot study lasted approximately two years and was the first field demonstration of a process developed by the University of Colorado in Brighton, Colorado from 1989 to 1991. Both towns are located in predominately rural eastern Colorado, rely on groundwater for their drinking water supply, and do not have the resources or skill necessary to operate a reverse osmosis drinking water plant.

The Wiggins study was conducted to operate a full-scale demonstration facility (with a capacity of 10 to 20 gpm), provide intensive monitoring over several seasons in order to obtain Colorado Department of Public Health approval, and evaluate process response to a variety of stresses and equipment failures. The results were outstanding.

The Wiggins study demonstrated the reliability and robust nature of the process. Once steady-state was established, the water nitrate levels fell from 20 – 25 mg/L in the raw water to a range of 2 to 4 mg/L as N. Further, it was found in the course of routine studies that after an upset the process could be revived quite quickly. For instance, when the carbon source feed pump failed, denitrification could be reestablished within 24 hours after restarting the pump.

One significant finding from the Wiggins study, however, was the choice of carbon source, or carbon substrate. Initially the researchers used food-grade acetic acid. Unfortunately, this substance proved to be hard to handle and quite expensive. The researchers then switched to food-grade corn syrup. The corn syrup was relatively inexpensive, could be added to potable water, and could be added using simple feed pumps. This option was adopted for future studies.

New York State Pilot Study

With the success at Wiggins, Nitrate Removal Technologies (NRT) of Golden, Colorado patented the process under the trade name *BioDen*. NRT pursued a number of demonstration studies using the technology, including one in Suffolk County, New York that EPRI sponsored. The aim of that study was discussed in detail above, but its principal goal was to demonstrate the efficacy of the technology for a large water utility.

During the testing, the four filter technologies were studied. During the Wiggins developmental testing, the researchers had used only slow sand filters. This study attempted to assess the efficacy of alternative, high-rate filtration options.

The pilot study demonstrated that the *BioDen* denitrification system consistently produced water with nitrate levels only 65 percent of the influent levels. Effluent from the BioDen reactors ranged from 1.9 mg/L to 6.8 mg/L but averaged 3.4 mg/L. The average raw water nitrate concentration was approximately 9 mg/L. When coupled with the tested filtration systems, the system produced safe and high-quality drinking water with regards turbidity, nitrate, nitrite, and common bacteriological levels such as HPC and fecal coliform.

While the bag-and-cartridge filter was an abysmal failure, the other microfiltration techniques proved to be very effective. Both microfiltration systems performed better than the slow sand filters in terms of turbidity removal, removal of coliform bacteria, chlorine demand in the filter effluent, and removal of HPC bacteria. On the other hand, the slow sand filter was more effective at removing both total organic carbon and reducing total trihalomethane formation potential (TTHMFP).

Given the biological nature of the top layer of slow sand filters, these results are not surprising. Apparently, some of the organic carbon present in natural waters was biologically degraded by the biomass present in the sand filter. Particle removal in the microfiltration systems is purely a mechanical matter, so any biological reduction would not be expected.

Clearly for those systems small enough and with the capability of maintaining a slow sand filter, this choice makes sense. However, for larger systems or for those drinking water systems in areas with limited land space, the microfiltration systems combined with the BioDen biological denitrification system is an effective means of producing potable water from groundwater supplies polluted with high levels of nitrates.

Proposed Modesto Pilot Study Protocol

On December 20, 2000, the City of Modesto officially notified researchers with Nitrate Removal Technology of its decision to delay the move forward on the Grayson Biological Denitrification Project. The City will pursue the possibility of installing a 1,000 foot deep well in the area of the Grayson system that might yield water below the nitrate action level. There has been considerable speculation the City staff are extremely reluctant to use microbes to accomplish a potable water treatment goal, even with disinfection of the effluent. This makes sense given that it is antiethical to water treatment professionals to encourage the growth of microbes during the treatment process. This problem is one that this particular technology must overcome before acceptance is widespread.

While EPRI and the researchers view this with acute disappointment, the team was able to secure approval from the California Department of Health Services for a suitable test protocol for this process. As designed, the protocol will yield valuable information for the Department to rule on the efficacy of the biological denitrification treatment system. Eventually, this will result in Department approval of the process for the removal of nitrates from potable water supplies in the State of California.

Based on discussions with California DHS and other interested parties, the Modesto pilot study will be conducted in two phases. The first phase will consist of a three-month demonstration of a 6 to 10 gpm pilot system. The principal goals of the assessment will be to evaluate water quality, and will be focused on the denitrification achieved and the impact on filtration. Included in this phase will be one month of biological perchlorate destruction. Perchlorate is an organic pollutant, commonly encountered in the Los Angeles basin, which is difficult to remove from drinking water with conventional technologies.

During Phase 2 of the study, a demonstration system capable of treating 300 gpm or more will be installed and operated. The demonstration system will be used to develop detailed cost data on the process. Preliminary estimates suggest that biological denitrification compares quite favorably to both ion exchange and reverse osmosis, which are the two technologies used to remove nitrates today.

While biological denitrification costs range between $0.55 and $1.40 per 1000 gallons, ion exchange costs from $ 0.55 to $ 1.85 and reverse osmosis costs range from $ 0.60 to $ 5.20 (both per 1000 gallons). The broad range in costs for the conventional treatment technologies is the result of brine disposal costs and electricity costs, which vary depending on the location. In California, these disposal costs and power costs are expected to be on the high side of these ranges. Further, given California's recent power issues, any technology which minimizes electricity costs, such as biological denitrification, may have inherent advantages over those which rely on electricity, such as reverse osmosis. That advantage is difficult to quantify; however, it is quite possible that a strict comparison of costs may not adequately address that difference.

GLOSSARY

Aerobic process	A biological process that requires oxygen for microorganisms to flourish.
Anaerobic process	A biological process that requires the total absence of oxygen so that fermentation can occur.
Anoxic process	A biological process that requires the total absence of molecular oxygen. Anoxic organisms can utilize oxidized inorganic compounds such as nitrites or nitrates.
Backwashing	The method used to clean filter media by reversing the water flow. Backwashing may be done in combination with compressed air.
Biochemical oxygen demand (BOD)	A measurement of the organic content of wastewater.
Chlorine residual	The amount of chlorine still available after a certain length of contact time with the water or wastewater.
Clarifier	A tank or basin used for the separation of suspended matter from the liquid phase by gravity settling. It is also called a sedimentation or settling tank.
Coagulation	The destabilization and initial aggregation of colloidal and finely divided suspended matter by the addition of a floc-forming chemical or by biological processes.
Cryptosporidium	A microorganism found in water supplies that causes a form of gastroenteritis.
Denitrification	The chemical reduction of nitrate to gaseous nitrogen. This chemical process can be accomplished biologically using nitrifying bacteria.
Disinfection	Destruction of disease causing microorganisms by physical or chemical means.
Filter media	The material through which water or wastewater is filtered.
Filtration	The process of passing a liquid through a filter to remove suspended solids.
Floc	Small jelly-like masses formed in a liquid by adding a coagulating chemical.
Flocculation	The collection of coagulated suspended solids into a mass by gentle stirring.
Giardia lamblia	A microorganism found in water supplies that causes a form of gastroenteritis.
Influent	Water, wastewater, or other liquid flowing into a reservoir, basin, or treatment plant, or any unit thereof.
Inorganic	Chemical substances of mineral origin, or more correctly, not of basically carbon structure.
Membrane filter	Technology used in water treatment for liquid-solids separation; system usually consists of forcing a liquid under pressure through a fine pore membrane capable of removing small-size contaminants from water.
Nitrification	The biological oxidation of ammonia to nitrate.

Nutrient	An element that is essential for the growth of plants and animals. Nutrients in wastewater, usually nitrogen and phosphorus, may cause unwanted algal and plant growths in lakes and streams.
Organic	Chemical substances of animal or vegetable origin, or more correctly, of basically carbon structure, comprising compounds consisting of hydrocarbons and their derivatives.
Ozone	An unstable gas consisting of three atoms of oxygen. Ozone is used as an oxidizing agent or a disinfectant.
Sedimentation	Settling or clarification; the process of allowing solids in water and wastewater to sink by gravity.
Solids	Material removed by water and wastewater treatment. Solids consist of organic and inorganic matter and water. Wastewater solids are residuals that exist before the biosolids portion has been treated to the point at which it is suitable for beneficial reuse.
Turbidity	A murkiness in water caused by suspended matter.

REFERENCES

Cook, N.E. and Silverstein, J. "Biological Denitrification of Polluted Groundwater" Completion Report No.153, Colorado Water Resources Research Institute, 1989, pp. 40-43.

Cook, N.E., Siverstein, J., Veydovec, B., de Mendoca, M.M., and Sydney, R. "Field Demonstration of Biological Denitrification of Polluted Grounwater and Pilot Scale Field Testing of Biological Denitrification With Widely Varied Hydraulic Loading Rates" Completion Report No. 162, Colorado Water Resources Research Institute, 1991, pp. 21-22.

Denn, J. "Heterotrophic Bacteria Debate Continues" *Water Technology*, Oct. 1999, pp. 62-63.

Geldreich, E.E., Nash, H.D., and Spino, D. "Characterizing Bacterial Populations in Treated Water Supplies: A Progress Report," *Proc. American Water Works Association Water Quality Technology Conf*., Kansas City, MO, December 1977.

Lin, Y.E., Vidic, R.D., Stout, J.E., and Yu, V.L. "Legionella in Water Distribution Systems" *Journ. A.W.W.A*, Vol. 90, No. 9, 1998, pp. 112 – 121.

Water Quality and Treatment: A Handbook of Community Water Supplies – 4th Edition, American Water Works Association, Pontius, F.W.-Editor, McGraw Hill, Inc., New York, 1990, pp. 1125-1126.

Opflow, "Emerging Water Quality Issues Survey, AWWA Emerging Water Quality Issues Committee Report", *Opflow*, Vol. 25, No. 10, October 1999, pp. 3,14.

Bodelier, P.L., Wijlhuizen, A.G., C. Blom, and Laanbroek, H.J. 1997. Effects of Photoperiod on Growth of and Denitrification by *Pseudomonas chlorophis* in the Root Zone of *Glyceria maxima*, Studied in a Gnotobiotic Microcosm. *Plant and Soil*. Vol. 190, pp. 91-103.

Han, S.O. and New, P.B. 1998. Isolation of *Azospirillum spp*. from Natural Soils by Immunomagnetic Separation. *Soil Biol. Biochem*. Vol 30, No. 8/9, pp.975-981.

Madigan, M.T., Martinko, J.M., and Parker, J. 1997. "Brock: Biology of Microorganisms, Eighth Edition". Prentice Hall, New Jersey., pp. 700-701.

Rosselló-Mora, R.A., Wagner, M, Amann, R., and Scleifer, K. 1995. The Abundance of *Zoogoea ramigera* in Sewage Treatment Plants. *Appl Environ. Microbiol.* Vol. 61, No. 2, pp. 702-707.

Strand, S.E., McDonnell, A.J., and Unz, R.F. 1988 Oxygen and Nitrate Reduction Kinetics of a Nonflocculating Strain of *Zoogloea ramigera. Antonie Van Leeuwenhoek.* Vol. 54, No. 3, pp. 245-255.